

## Evaluation Of Antidiabetic And Cytotoxic Effect Of Boerhavia Diffusa Mediated Selenium Nanoparticles

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### Abstract

**Introduction:** Boerhavia diffusa is a species of flowering plants, also known as the four o'clock family commonly called Punarnava. It is used as a herbal medicine for pain relief. Leaves of Boerhavia diffusa are used as green vegetables in most parts of the world. Chloroform extract of root and aerial parts of Boerhavia diffusa is used in ayurveda for the treatment of diabetes mellitus. Selenium nanoparticles play an important role in inhibition of bacterial growth at very low concentrations of protein, a high amount is required for inhibiting bacterial growth individually. They act on both the intact and complex form of the extract. These nanoparticles serve to reduce the toxicity, enhance bioactivity, and improve targeting.

**Aim:** The main objective of this study is to estimate the anti diabetic and cytotoxic activity of Boerhavia diffusa mediated through Selenium nanoparticles.

**Materials & Method:** Extract preparation- 1g of Boerhavia diffusa was added in 100ml of distilled water and is boiled for 10 - 15 minutes at 70 degree Celsius.

Brine shrimp lethality Assay: Number of dead nauplii / Number of dead + Number of live nauplii x 100.

In vitro diabetic Assay: %inhibition = C-T/Cx100, where C-control & T-test sample.

**Results:** Boerhavia diffusa has a significant antidiabetic and cytotoxic activity. It helps in blood glucose reduction by rejuvenation of pancreatic B cells or by extra pancreatic action, which is said to be its antidiabetic activity. From the results obtained, it is clearly seen that Boerhavia diffusa has a major antidiabetic activity and a moderate cytotoxic activity.

**Conclusion:** From the study it can be concluded that Boerhavia diffusa aids in the therapeutic management of chronic ailments. It has major anti diabetic and moderate cytotoxic activity. It helps in the treatment of diabetes and renal diseases. Further studies have to be done on other activities of Boerhavia diffusa.

**Keywords:** Boerhavia diffusa, Antidiabetic, Cytotoxic, Selenium nanoparticles, Chronic ailments, Diabetes Mellitus, Eco friendly.

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### Introduction

Boerhavia diffusa is a species of flowering plants, also known as the four o'clock family commonly called Punarnava. It is used as a herbal medicine for pain relief. Leaves of Boerhavia diffusa are used as green vegetables in most parts of the world (1). In addition to anti- diabetic and cytotoxic effects, it also possesses anti-inflammatory activity. It is a good cure for many diseases. It stimulates rheumatoid arthritis (2). Roots of Boerhavia diffusa are used as an anticonvulsant, analgesic and as a laxative medication (3). When these roots are rubbed with honey, it is applied for cataract, conjunctivitis. Consumption of leaves of Boerhavia diffusa reduces oedema. Oral administration of Boerhavia diffusa decreases the blood glucose level, increases

plasma insulin level (4). It also reduces the glycosylated haemoglobin level and increases the normal haemoglobin level (5). In diabetic patients, it increases the OGTT(Oral Glucose Tolerance Test). Methanolic extract of *Boerhavia diffusa* has a significant anti- diabetic property which is associated with the free radical scavenging and antioxidant activity (6).

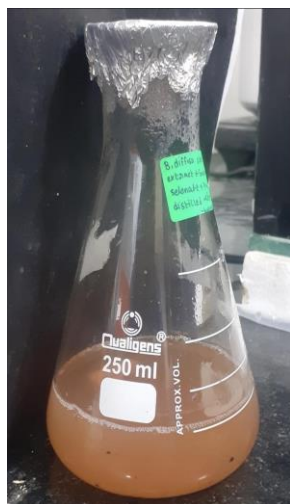
*Boerhavia diffusa* has an inhibitory effect on xanthine oxidase, lipoxygenase and anticholinesterase enzyme (7). Chloroform extract of root and aerial parts of *Boerhavia diffusa* is used in ayurveda for the treatment of diabetes mellitus (8). Other actions associated with *Boerhavia diffusa* are antibacterial, antinociceptive, antiproliferative, antiestrogenic, anti-inflammatory, anticonvulsant (9). It is also used in the treatment of stress, dyspepsia, abdominal pain, inflammation and jaundice (10). Crude extract of *Boerhavia diffusa* has remarkable antimicrobial screening against tested microorganisms (11), (12). Extracts of *Boerhavia diffusa* plant have significant inhibitory activity against *Candida Albicans*, which causes oral thrush (9,13). It comprises the cell membrane integrity, vital dyes and also it stains the intracellular components. Some research suggests that several nanoparticles have biomedical applications like copper nanoparticle, Silver nanoparticles, Likewise Selenium nanoparticle has also been used (14), (15).

Selenium is a non-metal with the properties that are intermediate between the elements in the periodic table. Selenium nanoparticles play an important role in inhibition of bacterial growth at very low concentrations of protein, a high amount is required for inhibiting bacterial growth individually (16). They act on both the intact and complex form of the extract (17). These nanoparticles serve to reduce the toxicity, enhance bioactivity, and improve targeting. Selenoproteins have oxidoreductase activity and thus regulate the physiological redox balance (18), (19). It constitutes an attractive carrier platform for various drugs to the site of action. (20–31),(32–36),(37) (38) (39). The aim of this study is to determine both the antidiabetic and cytotoxic effect of *Boerhavia diffusa* mediated by selenium nanoparticles.

## **MATERIALS AND METHOD:**

### **Preparation of extract:**

1g of *Boerhavia diffusa* was added in 100ml of distilled water. It was boiled for 10- 15 minutes at 70 degree celsius. After boiling, the plant extract was filtered using Whatmann's no.1 filter paper. In a 250 ml conical flask, 60 ml of 30 millimolar sodium selenite was prepared and 40 ml of the filtered plant extract was mixed. This flask was kept in a magnetic stirrer. The synthesised nanoparticles were preliminarily analysed by using UV visible spectrophotometer. The nanoparticle solution was centrifuged at 8000 rpm to prepare nanoparticle pellets. The nanoparticle pellet was dried in a hot air oven at 80 degree celsius. The dried powder was sent for keratinization.



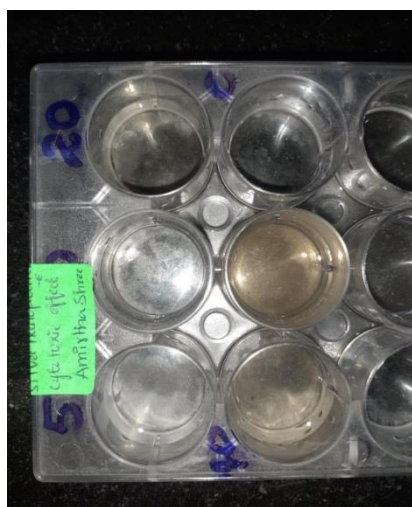
**Figure.1** This figure shows the filtered extract of *Boerhavia diffusa*.

**Cytotoxic activity (Brine Shrimp Lethality Assay):**

2g of iodine free salt was weighed and dissolved in 200 ml of distilled water. 6 well ELISA plates were taken and 10- 12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20 $\mu$ L, 40 $\mu$ L, 60 $\mu$ L, 80 $\mu$ L, control). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24hrs.

After 24hrs, the ELISA plates were observed and noted for number of nauplii present and calculated by using formula,

$$\text{Number of dead nauplii} / \text{number of dead nauplii} + \text{number of live nauplii} \times 100$$



**Figure.2** This figure shows the Brine shrimp lethality assay for the estimation of cytotoxic activity of *Boerhavia diffusa*.

**In- vitro diabetic assay:**

The in- vitro anti- diabetic assay was performed using two different techniques.

Alpha- amylase inhibitory assay and glucose diffusion- inhibitory assay.

**Alpha- amylase inhibitory assay:**

Alpha amylase inhibition was determined by quantifying the amount of maltose liberated during the experiment. The method reported by Bhutkar and Bhise has been followed. Different concentrations of nanoparticles (20, 40, 60, 80, 100 L) were pre- incubated with 100 L of alpha amylase solution (1U /mL) at room temperature for 30 minutes. 100 L of starch solution (1% w/v) was further added to it and the mixture was incubated at room temperature for 10 minutes. 100 L of 96mM (3,5 - dinitrosalicylic acid solution) DNSA reagent was added to it to stop the reaction and the solution was heated in a water bath for 5 minutes. Control was maintained where the equal quantity of enzyme extract was replaced by sodium phosphate buffer maintained at a pH value of 6.9. Reading was measured at 540 nm. The experiment was performed in triplicate. Acarbose was used as a positive. % inhibition was calculated using the formulae

$$\% \text{inhibition} = \frac{C - T}{C} \times 100, \text{ Where, } C = \text{control, } T = \text{test sample}$$

The results were analysed using SPSS software (Version 23). Statistical analysis was done using chi- square test, where the P value less than 0.05 was considered to be significant.

## RESULTS AND DISCUSSION

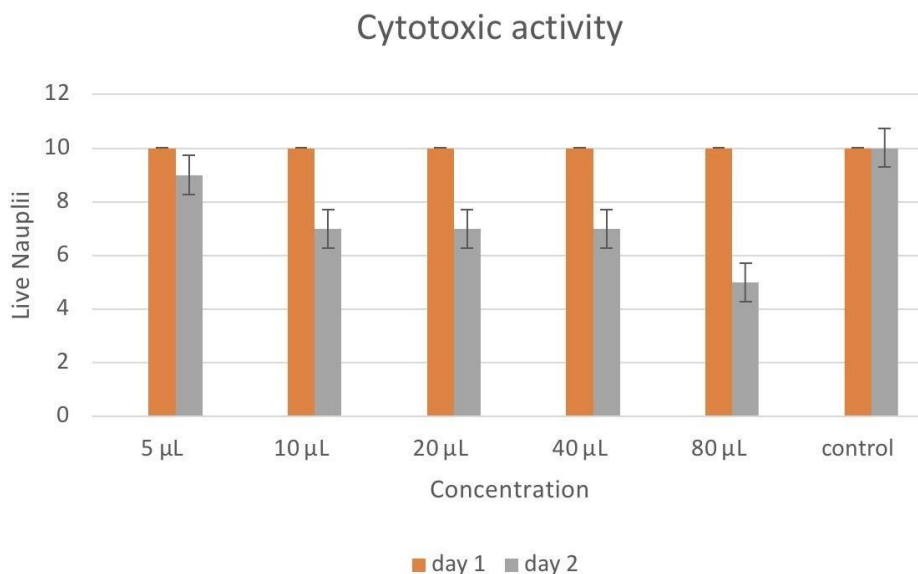
The results obtained are recorded and the percentage inhibition is calculated and shown in table 1 and table 2. Statistical analysis is done using Chi square test using SPSS software, where P value less than 0.05 is considered statistically significant.

Table.1 shows the cytotoxic activity of Boerhavia diffusa. In that, to each well of ELISA plate nauplii were added and selenium nanoparticles were added according to the concentration level. After 24 hours of incubation, nauplii were counted. Less concentration of nanoparticles doesn't kill much of the nauplii, whereas increase in concentration of nanoparticles kills more of nauplii. So using more of Boerhavia diffusa may cause side effects to humans but less than synthetic ones. Table.2 shows the antidiabetic activity of Boerhavia diffusa. 5 readings have been taken. Starch solutions with different concentrations of nanoparticles were added and analysed. Results show that, at 540 nm of wavelength, these five readings show different absorbent values and %T. Though Boerhavia diffusa possesses antidiabetic properties, at varying concentrations it acts differently in treating diabetes. From the results obtained, it is clearly seen that Boerhavia diffusa has a major antidiabetic activity and a moderate cytotoxic activity. By doing statistical analysis, the P value was found to be insignificant, so this plant can be used commercially. Cytotoxic activity may be due to the presence of alkaloids and also acts against the tumour cells(40), (4).

### CYTOTOXIC ACTIVITY:

**Table.1 This table shows the Concentration of extract acting against the Brine shrimp (nauplii) after 24 hours of incubation.**

Concentration	5µL	10µL	20µL	40µL	80µL	control
No.of nauplii	9	7	7	7	5	10



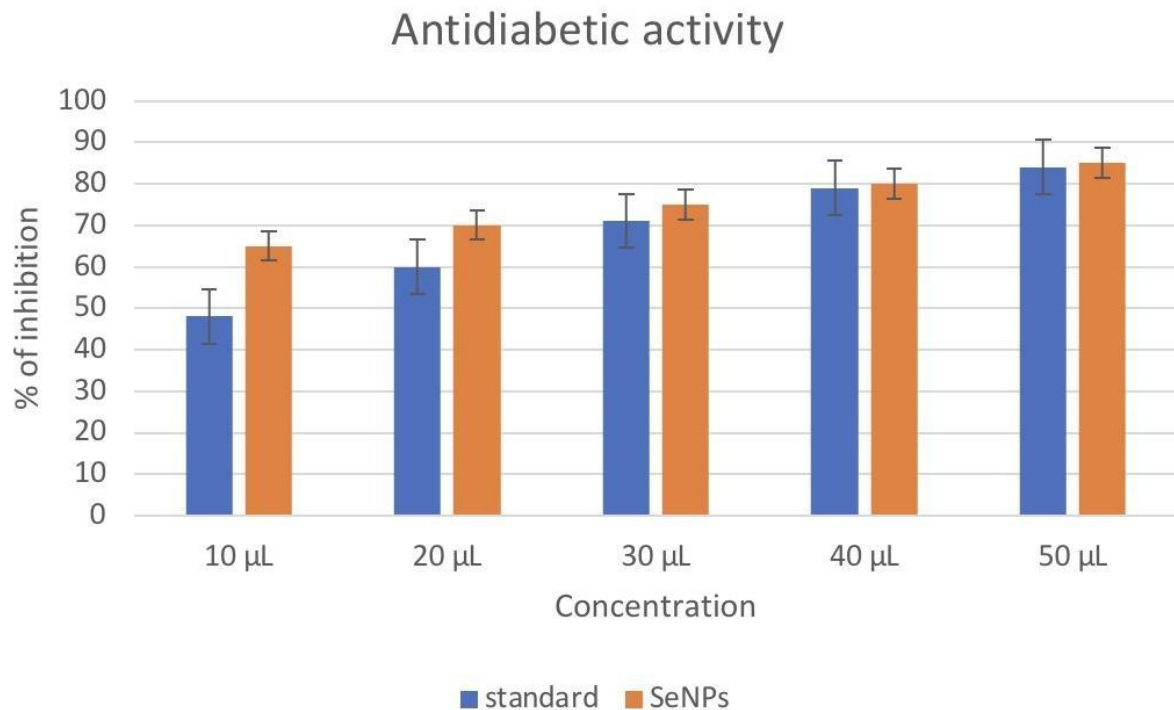
**Figure.4**

Bar graph depicts the cytotoxic activity of the *Boerhavia diffusa*. X- axis denotes the concentration of extract and Y- axis denotes the live nauplii in both day 1 and day 2. Orange colour represents the concentration on day 1 and Grey colour represents the concentration on day Chi square test was done and the P value was found to be 0.327 ( $P > 0.05$ ), which is statistically insignificant.

**ANTI DIABETIC ACTIVITY**

**Table.2** This table shows the antidiabetic activity of *Boerhavia diffusa* at the wavelength 540 nm. Also, the absorbance value and % inhibition values are mentioned

Concentration	Wavelength	Absorbent	% of inhibition
10μL	540	0.073	84.5
20μL	540	0.075	84.2
30μL	540	0.073	84.5
40μL	540	0.077	83.8
50μL	540	0.072	84.7



**Figure.5**

This Bar graph depicts the antidiabetic activity with standard errors of *Boerhavia diffusa* extract. In this the X- axis denotes the concentration of extract and Y- axis denotes the % of inhibition. Blue colour represents the standard and orange colour represents the Selenium nanoparticles. Chi square analysis was done and the P value was found to be 0.352, which is statistically insignificant.

*Boerhavia diffusa* has a significant antidiabetic and cytotoxic activity. It helps in blood glucose reduction by rejuvenation of pancreatic B cells or by extra pancreatic action, which is said to be its antidiabetic activity (41), (42). In ancient times, this plant was used in controlling diabetes mellitus (43). It also possesses immunomodulatory properties(44). Maximum diuretic effect is also observed in *Boerhavia diffusa* (45). By combining both the activities of *Boerhavia diffusa*, it is used to cure the chronic renal ailments in humans (46), (47).

By comparing with previous studies done on *Boerhavia diffusa*, it is said that methanolic extract of *Boerhavia diffusa* employs a significant antidiabetic activity in Wistar rats, which is associated with its free radical scavenging & antioxidant activity(48), (49). Another study which has been done by preparing the chloroform extract of *Boerhavia diffusa* concludes that it has antidiabetic activity and also it supports the traditional usage of the plant by Ayurvedic physicians for controlling diabetes (50). Also, ethanolic extract of *Boerhavia diffusa* possess mild cytotoxic and potent anti cancer activity (51). Limitations of this study include, only cytotoxic and anti diabetic activity of *Boerhavia diffusa* has been assessed. Also, only the leaf extract is used in this study. In future studies can be done with larger samples, other parts of the plant and many other activities can be estimated.

## CONCLUSION

It is concluded that *Boerhavia diffusa* aids in the therapeutic management of chronic ailments. It has major anti diabetic and moderate cytotoxic activity. It helps in the treatment of diabetes and renal diseases. Further

studies have to be done on other activities of *Boerhavia diffusa*, to use the extracts as commercial products. Though *Boerhavia diffusa* has so many pharmacological properties, it also has side effects.

## CONFLICT OF INTEREST

None declared

## AUTHOR CONTRIBUTIONS

Keerthana B: Literature research, analysis, manuscript writing.

Dr. R V Geetha: Study design, Manuscript drafting

Dr. S Rajesh Kumar: Provide guidance for undergoing research, data verification, manuscript correction.

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